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Effect of ionizing radiation on physiological function in the anesthetized rat

W. A. Alter III
G. N. Catravas
R. N. Hawkins
C. R. Lake

DEFENSE NUCLEAR AGENCY
ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
BETHESDA, MARYLAND 20814-5145

REVIEWED AND APPROVED

GEORGE N. CATRAVAS, D.Sc.
Chairman
Biochemistry Department

LAWRENCE S. MYERS, Ph.D.
Scientific Director

BOBBY R. ADCOCK
COL, MS, USA
Director

Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

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* from Uniformed Services University of the Health Sciences, Bethesda, Maryland

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Effect of Ionizing Radiation on Physiological Function in the Anesthetized Rat

WILLIAM A. ALTER III,* GEORGE N. CATRAVAS,†
ROBERT N. HAWKINS,† AND C. RAYMOND LAKE*

*Uniformed Services University of the Health Sciences, School of Medicine, 4301 Jones Bridge Road, Bethesda, Maryland 20814
†Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814

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Exposure of pentobarbital-anesthetized rats to 14.5-MeV electrons results in radiation-induced physiological dysfunction. Responses include transient hypotension, a transient decrease in heart rate, respiratory dysrhythmias, and a prolonged increase in pulse pressure. Magnitudes of these responses are dose related, and maximal responses can be elicited by either whole- or partial-body (head or abdominal) exposure to 10,000 rad. These responses were associated with a fivefold increase in arterial plasma concentration of epinephrine, whereas histamine, norepinephrine, and β-endorphin did not change during the first minute after the onset of exposure. Administration of diphenhydramine, a histamine receptor antagonist, resulted in a significant decline of baseline cardiovascular function and inhibited radiation-induced cardiovascular dysfunction. The diphenhydramine-induced decrease in preexposure blood pressure was reversed by angiotensin infusion, but this procedure failed to restore the mechanism(s) responsible for the cardiovascular responses to radiation. Results of these experiments and information available in the literature support the hypothesis that these responses are due to an interference in the autonomic pathways that modulate cardiovascular function.

INTRODUCTION

Exposure to significant doses of ionizing radiation can lead to acute disturbances in cardiovascular, respiratory, gastrointestinal, and neurologic function. Several animal species, including man (*1, 2*), monkey (*3, 4*), and rat (*5*), experience hypotension and performance decrement within minutes after exposure to ionizing radiation. One of the obvious consequences of radiation-induced hypotension could be underperfusion of the critical organs (e.g., heart and brain) that normally possess intrinsic and extrinsic mechanisms for maintaining tissue blood flow in the presence of moderately reduced blood pressure. If the hypotension is severe and/or the compensatory mechanisms have been compromised, then radiation-induced hypotension can be a major contributing factor to both performance decrement (*1, 2*) and the myocardial infarctions reported in patients after therapeutic partial-body radiation (*6*).

Beginning with the early work of Lewis (*7*), many investigators have proposed that histamine is the primary cause for several of the acute physiological responses to radiation. Histamine is a potent vasoactive substance that is found in most body tissues, but primarily within tissue mast cells and in circulating basophils. Effects on

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the vasculature include arteriolar dilation, venoconstriction, and increased capillary permeability (*8*), and it is these factors that may cause radiation-induced hypotension as well as erythema and edema.

Evidence to support this "histamine hypothesis" includes the finding that plasma levels of histamine rise postexposure (*9*) at a time that coincides with the onset of cardiovascular dysfunction (*3, 4*). In addition, pretreatment with the H_1-receptor antagonist chlorpheniramine partially inhibits radiation-induced performance decrement in monkeys (*10*) and rats (*11*). In contrast, pretreatment with 48/80, an agent that depletes mast cell stores (*12*), does not inhibit radiation-induced erythema in rats (*13*). These findings indicate that at least this species undergoes vascular responses that do not appear to involve histamine.

One of the primary mechanisms for maintaining systemic arterial blood pressure within the normal range involves activation of the sympathetic nervous system. In response to abrupt decreases in blood pressure or blood volume, increased sympathetic activity leads to cardioacceleration and peripheral vasoconstriction. It is proposed that mechanisms responsible for radiation-induced cardiovascular dysfunction may include dysregulation of sympathetic nervous system function. One of the methods available for assessing acute changes in sympathetic activity is measurement of plasma epinephrine and norepinephrine. Low levels of epinephrine and norepinephrine postexposure might indicate inhibition or damage, while high levels might indicate activation of sympathetic pathways. Based on results obtained from rats during endotoxic shock, it appears that the release of endorphins (an opiate peptide) impedes compensatory mechanisms by inhibiting sympathetic output from the brain (*14*).

This study was undertaken to determine the effects of radiation on physiological function in the anesthetized rat model. Experiments were also conducted to evaluate the effect of radiation on plasma levels of histamine, epinephrine, norepinephrine, and β-endorphin. Finally, pretreatment with antihistamines was used to determine if pharmacologic intervention could interfere with a mechanism(s) responsible for radiation-induced physiological dysfunction.

METHODS

Male Sprague–Dawley rats (300–400 g) served as the animal model for these experiments. Numbers of animals used for each series of experiments appear in the text or appropriate table. Each was anesthetized with pentobarbital (75 mg/kg, ip), and the trachea was intubated. A catheter was inserted into the femoral artery to measure arterial blood pressure and another into the femoral vein to administer drugs. Heart rate was computed electronically from the interval between blood pressure peaks. Body temperature was maintained at 37 ± 1°C by a feedback-controlled heat lamp, triggered by changes in rectal temperature. Respiratory activity was detected as changes in airway temperature from a nonobstructing thermocouple located in the tracheal tube.

Radiation exposures were conducted in the linear accelerator at the Armed Forces Radiobiology Research Institute. For these experiments, the electron beam was scattered through a water target, thereby providing an effective electron energy of 14.5 MeV to the animals. Dosimetry was conducted daily on a tissue-equivalent rat phantom, using both ionization chambers and thermoluminescent dosimeters.

To characterize the acute physiological responses, different groups of rats underwent whole-body exposures at doses of 1000 to 40,000 rad. At a dose of 10,000 rad, the effects of dose rate (180 to 1200 rad/sec) were also investigated. To determine the body regions most critical to these responses, other groups underwent partial-body radiation exposures (10,000 rad), in which either the midhead or midabdomen served as the center of a radiation field 5.0 cm long by 5.0 cm high. Remaining portions of the body were protected

behind a lead and paraffin shield, which reduced the effective dose at the midthorax to less than 1% of the target dose. Repeated partial-body or whole-body exposures were used to determine if the mechanism(s) involved in these responses remained intact or was at least temporarily inactivated by an initial exposure.

To determine the effect of radiation on plasma levels of vasoactive factors, arterial blood samples were obtained from rats that were connected to a reservoir (Fig. 1). A peristaltic pump withdrew blood from a carotid artery catheter into the reservoir and then returned blood into the femoral vein at a flow rate of 4 ml/min. Before starting the pump, the experimental rat was heparinized, and the reservoir was filled with 30 ml of blood drawn by aortic puncture from anesthetized donor rats. Once the exchange transfusion began, 15 min were allocated for mixing blood from the donor rats with the experimental rat's blood. Arterial blood samples were drawn from the reservoir input line while output to the animal continued. This permitted the withdrawal of five samples at the expense of reservoir residual volume while the experimental rat's blood volume was maintained relatively constant. Each blood sample was drawn over 1 min at 10 min before exposure and at 0, 10, and 30 min after the onset of exposure. Each animal then received 48/80 (iv) at 32 min postexposure to evaluate the integrity of mast cell stores of histamine. A final blood sample was drawn 5 min after this final injection. The zero time sample consisted of blood that had left the animal at the onset of radiation. By increasing the dose rate to 1200 rad/sec for this series of experiments, radiation was completed within 9 sec, thus making it possible for an investigator to enter the exposure room and obtain this zero time sample before it entered the reservoir.

One milliliter of arterial blood was immediately separated from each sample and used to determine arterial blood gases (pO_2, pCO_2) and pH to ensure that spontaneous respiratory activity provided adequate gas exchange. The remaining blood was kept on ice until it was centrifuged (within 20 min); then the plasma was removed and prepared for the study of selected vasoactive factors. All plasma samples were kept frozen at −70°C until analyzed. Vasoactive factors analyzed in this study (with name of technique in brackets) included histamine-like activity [spectrofluorometric (*15*)], epinephrine and norepinephrine [radioenzymatic (*16*)], and β-endorphin-like activity [radioimmunoassay (*17*)]. Several hematologic and

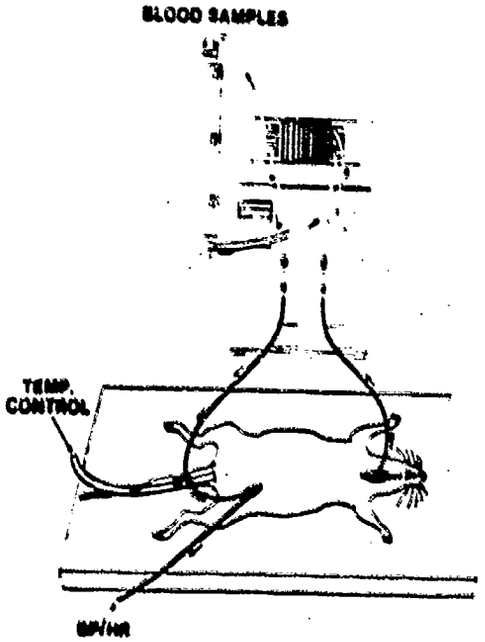

FIG. 1. Recirculation technique for repeated blood sampling from an anesthetized rat. Carotid arterial blood was pumped into a reservoir while blood was returned into the femoral vein at the same rate (4 ml/min). Reservoir was filled initially with arterial blood from donor animals. Blood samples were obtained from the reservoir input line.

metabolic parameters were measured to ensure that use of the peristaltic pump and extracorporeal reservoir did not adversely affect the blood, thereby producing an unstable preparation. Each of these parameters was measured in arterial blood obtained from the irradiated rats ($N = 7$) and from another group ($N = 6$) that underwent sham exposure. Metabolic status was assessed by measuring plasma levels of glucose [automated analyzer (*18*)], lactate and pyruvate [spectrophotometric (*19*)], and plasma protein [spectrophotometric (*20*)]. Standard laboratory procedures were used to measure hematocrit, hemoglobin concentration, red blood cell (RBC) fragility, RBC concentration, white blood cell (WBC) concentration, and a differential WBC count.

To determine whether pharmacologic intervention could inhibit the physiological responses to radiation, groups of rats received intravenous injections of H_1-receptor antagonists alone, or this was given in combination with 10 mg/kg of an H_2-receptor antagonist, cimetidine. Effectiveness of receptors' blockade was evaluated in preliminary experiments in which the dose–response curve for the blood pressure response to histamine was obtained before and after administration of these drugs. For the radiation experiments, H_1-antagonist doses selected were those that resulted in at least a 100-fold increase in the histamine dose required to cause a half-maximum hypotensive response (chlorpheniramine, 20 mg/kg; diphenhydramine, 10 mg/kg; pyrilamine, 10 mg/kg).

Results obtained in these studies were analyzed for statistical differences between baseline and postradiation values by using Student's t test for paired data with the Bonferroni modification (*21*) whenever repeated comparisons with the baseline value were made within groups. Comparisons between different groups of rats were made by using Student's t test for unpaired data. Data presented in this report are mean values and the standard errors of the mean.

RESULTS

In the initial series of experiments, pentobarbital-anesthetized rats had baseline physiological values of 119 ± 6 mm Hg for mean arterial pressure, 39 ± 2 mm Hg for pulse pressure, 395 ± 14 beats/min for heart rate, and 79 ± 6 breaths/min for respiratory rate. Groups of rats were exposed to 14.5-MeV electrons at doses ranging from 1000 to 40,000 rad. Threshold for acute physiological dysfunction was between 1000 and 2000 rad, and irradiation resulted in a transient decline in blood pressure. Figure 2 shows the effect of dose on the percentage decrease in mean arterial pressure recorded during the first minute after the onset of irradiation. Magnitude for radiation-induced hypotension reached a maximum level after 5000 rad.

Typical responses to radiation obtained in these studies are shown in Fig. 3A. Exposure to 10,000 rad resulted in a hypotensive episode (32 ± 4%) that was accompanied by a small but consistent decrease in heart rate (7 ± 4%). Only the decrease in mean arterial pressure was significantly different ($P < 0.01$) from baseline values. Both mean arterial pressure and heart rate returned to baseline levels within 2 min after the onset of exposure, and at this time an increase in pulse pressure and a decrease in respiratory rate became evident. In this group that received 10,000 rad, pulse pressure rose by 21 ± 3%, whereas respiratory rate fell by 28 ± 6% by 5 min after the onset of exposure. Both increases in pulse pressure and respiratory dysfunction were greater at doses above 10,000 rad. For example, rats that received 40,000 rad ($N = 6$) experienced a hypotensive response (34 ± 5%) that was followed by a 45 ± 6% increase in pulse pressure after 5 min, whereas respiratory rate fell by 48 ± 18%; in addition, respiratory tracings indicated that most animals were also dyspneic.

Effect of dose rate on radiation-induced physiological dysfunction was also investigated by exposing rats to 10,000 rad over a range of 180 ($N = 7$) to 1200 rad/sec ($N = 8$). An example of responses obtained at the highest dose rate is shown in the lower panel of Fig. 3B. Magnitude of the hypotensive response is not significantly different from that observed at the lowest dose rate. However, an increase in pulse

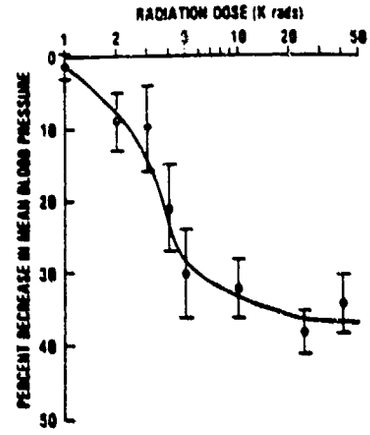

FIG. 2. Effects of radiation dose on percentage decrease in mean arterial pressure of anesthetized rats. All exposures were to 14.5-MeV electrons delivered at a rate of 180 rad/sec. Doses used were 1, 2, 3, 4, 5, 10, 25, and 40 krad. Data points represent $\bar{x} \pm$ SEM for groups of six or more animals.

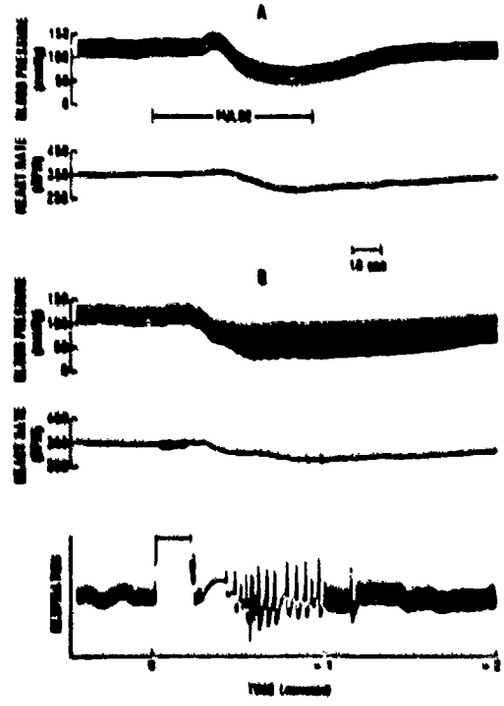

FIG. 3. Typical cardiovascular responses to 10,000 rad of 14.5-MeV electrons delivered at a dose rate of (A) 180 or (B) 1200 rad/sec. Also shown in (B) is the respiratory activity detected by a nonobstructing thermocouple located in the tracheal tube. Radiation exposure is denoted in (B) by the square wave deflection in this tracing.

pressure (50 ± 10%) during the hypotensive phase was evident in animals exposed at the highest dose rate. The degree of respiratory dysfunction was also more severe, and included episodes of apnea and dyspnea within 30 sec after the onset of exposure. As can be seen in the respiratory activity tracing in Fig. 3B, a brief period of apnea followed by dyspnea was evident within the first minute after the onset of exposure. Although respiratory rhythm recovered, rate declined by 48 ± 18% at 5 min post-exposure and then gradually returned to preexposure levels over the next 10 min.

To determine the body regions most critically involved in initiating the hypotensive response, groups of rats received partial-body radiation, and the results were compared to animals that received whole-body radiation. A minimum of seven animals was used in each group; results are summarized in Table I. All groups had similar levels of mean arterial pressure before radiation. One group of rats received an initial dose of 10,000 rad to the head region, and this resulted in a 27 ± 5% decrease in mean arterial pressure. Repeating this exposure after 10 min failed to elicit significant changes in any of the measured physiological parameters. Subsequent exposure of the abdominal region in this same group, however, elicited a hypotensive response (35 ± 6%) that was not significantly different from that observed for the initial head exposure. Repetition of this abdominal exposure failed to produce any physiological responses. A second group of rats received abdominal exposure before head exposure. Magnitudes of radiation-induced hypotension recorded for the initial regional exposures (abdominal = 34 ± 7%; head = 33 ± 6%) in this second group were not significantly different from those described for the first group that had received head irradiations before abdominal irradiations. Table I also shows that repeated whole-body exposures 10 or 30 min after an initial whole-body exposure failed to produce any significant physiological responses.

TABLE I

Effects of Repeated Exposures to 10,000 rad on the Acute Hypotensive Response of Anesthetized Rats[a]

Group	N	Baseline mean arterial pressure (mm Hg)	Percentage decrease in mean arterial pressure			
			1st Pulse	2nd Pulse	3rd Pulse	4th Pulse
Whole body (10-min intervals)	7	119 ± 6	−32 ± 3	−5 ± 2	−2 ± 1	−2 ± 1
Whole body (30-min intervals)	7	125 ± 8	−38 ± 4			−2 ± 6
			Head	Head	Abdomen	Abdomen
Head–Abdominal	10	123 ± 6	−27 ± 5	−2 ± 2	−35 ± 6	−3 ± 2
			Abdomen	Abdomen	Head	Head
Abdominal–head	7	123 ± 9	−34 ± 7	−2 ± 4	−33 ± 6	−5 ± 4

Note All data are $\bar{x}$ ± SEM.

[a] Interval between exposures was 10 min except for animals in the second whole-body-irradiated group which were irradiated 30 min after the initial exposure.

Blood samples obtained from rats undergoing continuous exchange transfusion (Fig. 1) revealed that radiation elicited prompt changes in several parameters, which coincided with cardiovascular and respiratory dysfunction. Before exposure, one group of rats ($N = 7$) had a respiratory rate of 73 ± 4 breaths/min, which provided adequate arterial blood gases (pO_2 = 74.7 ± 5.4 mm Hg; pCO_2 = 47.6 ± 2.1 mm Hg) and pH (7.33 ± 0.02). Sham-irradiated rats ($N = 6$) had a higher baseline respiratory rate (83 ± 6 breaths/min). This was associated with a similar arterial pO_2 (77.8 ± 4.1 mm Hg) but a lower pCO_2 (39.6 ± 4.5 mm Hg) and a higher pH (7.38 ± 0.2) than recorded in the irradiated group. Exposure to 10,000 rad resulted in respiratory dysfunction, which included a trend toward a decline in rate to 52 ± 8 breaths/min during the period of radiation-induced hypotension. However, this did not result in hypoxia (pO_2 = 77.7 ± 7.9 mm Hg) or acidosis (pCO_2 = 41.0 ± 3.0 mm Hg; pH = 7.36 ± 0.03). There were also several incidences of respiratory arrhythmias during the first few moments postexposure. Rate remained depressed even 10 min postradiation (58 ± 4 breaths/min), but blood gases and pH were not significantly different from those measured immediately postradiation. There were no significant changes in respiratory function in the sham-irradiated group over this same interval.

Baseline plasma glucose levels in the irradiated and sham-irradiated groups were 145 ± 8 and 133 ± 5 mg%, respectively. Radiation resulted in a gradual increase that reached 241 ± 17 mg% by 30 min postradiation. This value was significantly different from the baseline value in the irradiated group ($P < 0.01$), and was also somewhat (but not significantly) greater than glucose levels (174 ± 21 mg%) obtained in the other group at a similar time after sham exposure.

Hematologic data revealed that lymphocyte concentrations were 82.3 ± 3.1% in the irradiated group and somewhat higher in the sham-irradiated group (90.8 ± 2.6%). By 30 min postexposure there was a significant ($P < 0.01$) decline in percentage lymphocytes, which reached 73.1 ± 1.5% in the irradiated group. In contrast, this parameter did not change in the sham-irradiated group. None of the other metabolic or hematologic parameters that were measured showed any significant change over the course of these experiments.

In the irradiated group attached to the reservoir, baseline mean arterial pressure was 114 ± 7 mm Hg and heart rate was 385 ± 14 beats/min. Exposure to 10,000 rad resulted in a significant ($P < 0.01$) hypotensive response (36 ± 3%) and a small decrease in heart rate (3 ± 1%). Blood pressure results and plasma levels of the four vasoactive factors measured in these experiments are summarized in Table II. Arterial blood obtained during the first minute after the onset of irradiation revealed that norepinephrine, β-endorphin, and histamine did not change significantly, whereas epinephrine rose fivefold. Also there was a highly significant ($P < 0.001$) negative correlation ($r = -0.795$) between the change in the logarithm of epinephrine and mean arterial pressure. Over the postexposure period, mean arterial pressure, histamine, and β-endorphin remained near baseline values, whereas epinephrine remained elevated and norepinephrine rose to peak values at 10 min but then subsided somewhat by 30 min. Within the sham-irradiated group, mean arterial pressure decreased somewhat over the experimental period, and there was a gradual increase in plasma epinephrine, while values of the other vasoactive factors remained near baseline values.

Animals in both groups received 1.0 mg/kg of 48/80 to evaluate the integrity of

TABLE II

Effect of 10,000 rad on Mean Arterial Pressure and Plasma Levels of Vasoactive Factors

	Group	Time (min)				
		−10	0 to 1	+10	+30	+37[a]
		RAD				48/80
Mean arterial pressure (mm Hg)	R	114 ± 7	72 ± 3[b,c]	109 ± 6	113 ± 4	78 ± 4[b]
	S-R	119 ± 6	124 ± 5	105 ± 4	105 ± 11	73 ± 10[b]
Histamine (ng/ml)	R	34 ± 6	29 ± 4	23 ± 2[b]	28 ± 5	989 ± 91[b]
	S-R	21 ± 4	23 ± 4	19 ± 3	21 ± 4	1372 ± 288[b]
Epinephrine (pg/ml)	R	112 ± 52	530 ± 106[b,c]	291 ± 99	685 ± 146[b]	3218 ± 432[b]
	S-R	98 ± 42	116 ± 52	199 ± 82[b]	340 ± 116[b]	2505 ± 175[b]
Norepinephrine (pg/ml)	R	130 ± 23	141 ± 17	332 ± 89[b,c]	290 ± 63	476 ± 70[b]
	S-R	108 ± 22	116 ± 24	104 ± 18	139 ± 33	385 ± 49[b]
β-Endorphin (pg/ml)	R	2462 ± 278	2691 ± 701	2597 ± 362	2040 ± 339	4000 ± 680[b]
	S-R	2513 ± 294	2613 ± 582	2267 ± 419	3146 ± 732	5633 ± 1384

Note. All values are $\bar{x} \pm$ SEM. R = Irradiated group ($N = 7$). S-R = Sham-irradiated group ($N = 6$).

[a] Data were obtained 5 min after administration of 1.0 mg/kg of 48/80.

[b] Significantly different from pretreatment (radiation or 48/80) value ($P < 0.01$).

[c] Significantly different from sham group value ($P < 0.01$).

mast cell stores of histamine. For the irradiated group, there was a 35-fold increase in histamine, which resulted in a marked decrease in mean arterial pressure (26 ± 5%), while pulse pressure increased (22 ± 5%) and respiratory rate decreased (14 ± 7%) by 5 min after 48/80. Associated with these changes were a fivefold increase in epinephrine and approximately twofold increases in norepinephrine and β-endorphin. Administration of 48/80 to the sham-irradiated group increased plasma histamine to a level that was not significantly different from that measured in the irradiated group. In addition, cardiovascular and respiratory responses were not significantly different from those obtained in the irradiated group. There were also comparable changes in plasma epinephrine, norepinephrine, and β-endorphin.

Based on these results, it appears that postexposure physiological dysfunction is not a consequence of radiation-induced release of histamine. It is possible, however, that irradiation did result in an increase in histamine that was locally effective on the vasculature, but that it was then metabolized too rapidly for this technique to detect changes in arterial plasma levels. A second possibility is that the assay was not sensitive enough to detect plasma levels of histamine that cause radiation-induced physiological dysfunction.

To evaluate the first possibility, a second series of reservoir experiments were conducted in which experimental rats ($N = 8$) were pretreated with aminoguanidine to inhibit histaminase (*22*). In addition, these rats were artificially respired at a rate of 92 ± 2 breaths/min and at a tidal volume of 3.0 ml, which increased arterial pO_2 (87.2 ± 5.4 mm Hg). But this was not significantly greater than that measured in the spontaneously respiring group (74.7 ± 5.4 mm Hg). Baseline values for mean arterial pressure (107 ± 5 mm Hg), histamine (28.7 ± 3.1 ng/ml), and epinephrine (100 ± 22

pg/ml) were not significantly different from the values shown in Table II, whereas norepinephrine (189 ± 25 pg/ml) was significantly higher ($P < 0.05$) in the artifically respired group. Plasma levels of β-endorphin and the other hematologic and metabolic parameters were not measured in these experiments. Exposure to 10,000 rad elicited a hypotensive response that was similar in magnitude (40 ± 5%) to those responses observed in earlier experiments. Once again, plasma epinephrine increased fivefold, whereas neither histamine nor norepinephrine changed during this response. These results indicate that pretreatment with aminoguanidine did not reveal any increase in plasma levels of histamine during radiation-induced physiological dysfunction.

Yet to be answered was the question of whether the technique was sensitive enough to detect increased plasma histamine that can cause these hypotensive episodes. To evaluate this issue, a final series of reservoir experiments was performed on rats ($N = 3$) that received aminoguanidine and were artificially respired. These rats received an intravenous infusion of histamine at a manually adjusted rate to produce a hypotensive response (Fig. 4) similar to that recorded from irradiated rats (see Fig. 3A). Arterial blood samples obtained during the hypotensive response revealed a fivefold increase in the plasma histamine. In addition, a small increase in heart rate was recorded during histamine-induced hypotension.

Experiments described up to this point do not support the hypothesis that histamine is responsible for radiation-induced physiological dysfunction in the rat. This appears to contradict available data from other species indicating that radiation induces an increase in plasma levels of histamine and that pretreatment with antihistamines inhibits many of the acute physiological responses to radiation (*9–11*). To evaluate the effectiveness of antihistamines in rats, groups were pretreated with combinations of histamine receptor antagonists before exposure (Table III) to 10,000 rad. Untreated rats (Group I) experienced a 30 ± 6% decline in mean arterial pressure after irradiation. This was accompanied by a small decrease in heart rate (9 ± 5%), whereas respiratory rate declined (14 ± 4%) and pulse pressure rose (25 ± 3%) by 5 min postexposure.

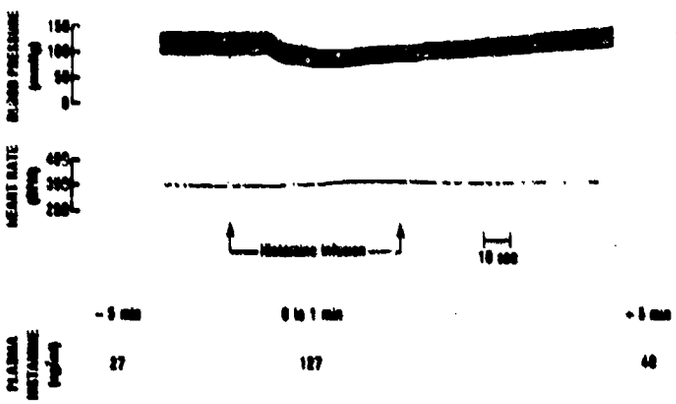

FIG. 4. Typical cardiovascular response to an intravenous histamine infusion. Plasma histamine levels were measured in arterial blood obtained from an anesthetized rat (pretreated with aminoguanidine) that was connected to a blood reservoir.

TABLE III

Effect of Histamine Receptor Antagonists on the Mean Arterial Blood Pressure (mm Hg) Response to 10,000 rad

Group	*N*	*Baseline*	*Postdrug*	*Angiotensin and respirator*[a]	*10,000 rad*
I. Untreated	7	118 ± 9			81 ± 8
II. Chlorpheniramine + cimetidine	7	127 ± 11	110 ± 10[b]		94 ± 7
III. Diphenhydramine + cimetidine	7	130 ± 7	98 ± 8[b]		96 ± 6
IV. Diphenhydramine + cimetidine	7	134 ± 2	90 ± 6[b]	136 ± 6	141 ± 7
V. Diphenhydramine	12	115 ± 4	95 ± 5[b]	118 ± 5	112 ± 5
VI. Cimetidine	6	115 ± 8	112 ± 9		69 ± 5[b]
VII. Pyrilamine + cimetidine	6	132 ± 9	94 ± 9[b]	134 ± 6	136 ± 5
VIII. Angiotensin + respirator	8	112 ± 4		146 ± 6	113 ± 4[b]

Note. Chlorpheniramine, 20 mg/kg; diphenhydramine, 10 mg/kg; pyrilamine, 10 mg/kg; cimetidine, 10 mg/kg. All data are $\bar{x} \pm$ SEM.

[a] Angiotensin infusion (0.06 ± 0.02 μg/min) and respirator were used to restore and maintain cardiovascular and respiratory function after antihistamines were administered.

[b] Significant decrease ($P < 0.01$) from the previous mean arterial pressure value within group.

This pattern of radiation-induced physiological dysfunction was similar to that described in the earlier experiments. Pretreatment with chlopheniramine and cimetidine (Group II) reduced by one-half the magnitudes of the hypotensive response (14 ± 3%) and the increase in pulse pressure (11 ± 3%) while the respiratory rate still decreased (11 ± 3%) an amount comparable to that recorded in the untreated group (I). Pretreatment with diphenhydramine and cimetidine (Group III) completely abolished the hypotensive response; in fact, mean arterial pressure rose gradually (12 ± 8%) over the 10-min postexposure observation period. This group also failed to show any decrease in respiratory rate, but the increase in pulse pressure (28 ± 5%) at 5 min postexposure was comparable to that recorded in the untreated group (I). Neither of these antihistamine-treated groups (II or III) experienced any significant decrease in heart rate during the immediate postradiation period.

One possible explanation for the increased effectiveness of diphenhydramine over chlorpheniramine (both were used in combination with cimetidine) is that the former had a greater effect on baseline cardiovascular function. The depressions in mean arterial pressure (22 ± 5%) and heart rate (20 ± 3%) induced by diphenhydramine were almost twice as great as those induced by chlorpheniramine (13 ± 3% and 13 ± 4%, respectively). Both combinations of drugs produced similar depressions in respiratory rate (approximately 10%). To determine if these reductions in function were responsible for inhibition of radiation-induced physiological dysfunction, diphenhydramine and cimetidine were administered to another group of rats (IV), but ventilation was maintained by a respirator and blood pressure was maintained by an infusion of angiotensin (0.06 ± 0.02 μg/min). Despite these efforts, heart rate remained 18 ± 2% below the level present before any antihistamines were administered, and, most importantly, radiation still failed to elicit any hypotensive response.

To determine which of the histamine receptor antagonists was primarily responsible

for blockade of radiation-induced hypotension, rats were pretreated with either diphenhydramine or cimetidine. Once again, pretreatment with diphenhydramine (Group V) induced a significant decline in mean arterial pressure and heart rate; therefore, these animals received angiotensin and were artificially respired. Radiation did not elicit any physiological dysfunction in this group. In contrast, pretreatment with cimetidine alone (Group VI) did not affect baseline cardiovascular function, and radiation elicited a hypotensive response (38 ± 3%) somewhat greater than that observed in the untreated group. To determine if the inhibition of radiation-induced physiological dysfunction was unique to diphenhydramine, another H_1 antagonist, pyrilamine, was administered in combination with cimetidine (VII). This reduced the preexposure values for mean arterial pressure and heart rate; therefore, these rats also received angiotension and were artificially respired. Exposure of Group VII failed to elicit any hypotensive response. To insure that angiotensin was not responsible for these results, rats in Group VIII were artificially respired and received angiotensin. Irradiation of this group elicited a 22 ± 2% decline in mean arterial pressure while heart rate decreased 5 ± 1%. As in the previous irradiation groups, both parameters recovered to baseline levels within 2 min.

DISCUSSION

These experiments indicate that irradiation of the pentobarbital-anesthetized rat results in prompt disruption in physiological function. Cardiovascular responses include a marked drop in mean arterial pressure and a small but consistent decrease in heart rate. Both parameters usually return to baseline values within 2 min postexposure. In addition, pulse pressure usually increased and remained elevated up to at least 10 min postexposure. Under conditions of this study, the threshold for the cardiovascular responses was between 1000 and 2000 rad, with maximum responses recorded at doses of 5000 rad or more. Respiratory function undergoes a consistent pattern of decreased rate, whereas episodes of apnea and dyspnea occur irregularly; however, the respiratory dysrhythmias appear to have an increased incidence at doses above 10,000 rad.

Characteristics of the cardiovascular responses to radiation in the rat are different from those reported for other species. Both man (*1, 2*) and monkey (*3, 4*) undergo a severe and prolonged hypotensive episode after exposure to doses above 1000 rad. Onset of this response is delayed somewhat, and minimum blood pressures are far lower in the monkey (*4*) than in the rat. In the present study, mean arterial pressure decreased by 25 to 40% in response to radiation, but it never fell to the extent that was reported in monkeys (*3, 4*). Earlier studies on the cardiovascular responses to radiation in the rat (*23*) did not report any acute hypotensive phase; however, radiation doses and dose rates were lower than those used in the present study. More recently, Mickley (*24*) reported that conscious rats experience a decline in tail artery pressure that coincides with performance decrement during the first 30 min after receiving 10,000 rad of 14.5-MeV electrons. It is not clear how changes in tail artery pressure after radiation relate to aortic pressure, because it has been shown (*25*) that the former decreases markedly in the irradiated rat at a time when aortic pressure is unchanged. Results of the present study also show that after the brief hypotensive phase, blood

pressure measured in the femoral artery is not depressed during the first 30 min postexposure. It is possible that these changes in tail artery pressure after radiation may be related to disturbances in thermoregulatory function in the rat, since the tail serves as an integral part of this process. This explanation is supported by the findings of acute disturbances in temperature regulation in the irradiated rabbit (*26*).

Most of the evidence accumulated in the literature indicates that histamine plays an important role in the acute physiological responses to radiation. Plasma histamine levels increased markedly within minutes after monkeys received 4000 rad of γ radiation (*9*), and pretreatment with chlorpheniramine partially inhibited both radiation-induced hypotension and performance decrement in this same species (*10*). Results of the present study indicate that radiation does not produce any detectable increase in plasma histamine for at least 30 min. Furthermore, the inability of radiation to cause a hypotensive response when either partial-body or whole-body exposures were repeated suggests that the active factor(s) present in the head and abdominal regions was depleted by the initial exposures. It is apparent, however, that tissue stores of histamine were not depleted by radiation, because administration of 48/80 within 30 min after radiation produced a marked increase in plasma histamine and a decrease in mean arterial pressure. These changes were comparable to those recorded from sham-irradiated rats.

Differences in responses between the rat and the monkey after high doses of radiation are somewhat surprising because both species undergo severe and prolonged hypotensive responses after administration of endotoxin [rat (*27*); monkey (*28*)]. In the monkey, cardiovascular responses to both radiation and endotoxin may be mediated by similar mechanisms, because it was found that pretreatment with sublethal radiation protected against the acute responses to endotoxin (*29*). The transient nature of the hypotensive episode after radiation in the rat versus the prolonged response to endotoxin (*27*) suggests that the causal mechanisms for these responses may be different in this species.

Other possible candidates for a role in the acute physiological responses to radiation include opiate peptides (*27*), bradykinin (*28*), and prostaglandins (*30*). All have been implicated in the physiological changes associated with endotoxic and anaphylactic forms of shock. In the present study, plasma levels of one opiate peptide, β-endorphin, were found to be markedly increased over levels reported in conscious rats (*16*), and radiation failed to alter these levels, whereas 48/80 doubled the concentration of β-endorphin. These results do not support the hypothesis that β-endorphin plays an important role in these responses to radiation, but it must also be stated that this evidence does not eliminate the possibility that other opiate peptides are involved.

Both bradykinin (*31*) and prostaglandins (*32*) satisfy many of the criteria required for factors that may be responsible for the physiological responses to radiation in the rat. Both are widely distributed throughout the body, are potent vasodilators, and are rapidly metabolized. Future studies will be directed toward determining the possible role of these factors in radiation-induced physiological dysfunction.

Another possible explanation for these responses involves a radiation-induced depression of sympathetic nerve influence on the cardiovascular system. Evidence is available that the baroreceptor reflex is impaired during the period of radiation-induced hypotension in monkeys (*4*). Radiation exposure of the rat may lead to a

transient disruption in autonomic activity, resulting in the hypotensive episode. If these pathways were not affected by radiation, this magnitude of hypotension should have led to a reflex increase in heart rate (as was recorded during histamine infusion, see Fig. 4) instead of the decrease that was consistently observed in these experiments.

Inhibition of radiation-induced cardiovascular dysfunction by H_1-receptor antagonists may be related to their effects on these same autonomic pathways. Antihistamines are well known for their ability to depress heart rate and blood pressure (*33*). This appears to be due to an anticholinergic affect (*34*) at the level of the autonomic ganglia. In the present study, angiotensin was used to restore the vascular tone (Table III) that was depressed by diphenhydramine and pyrilamine. Despite this effort, radiation failed to elicit physiological dysfunction, which indicated that restoration of blood pressure was not the key factor needed to reestablish radiation-induced cardiovascular dysfunction. This finding is not unexpected when it is considered that angiotensin acts on the peripheral vasculature and would not have directly affected antihistamine-induced depression in sympathetic activity. This contention is supported by the finding that angiotensin restored blood pressure but did not affect heart rate, which remained depressed in the antihistamine-treated rats. In rats that did not receive antihistamines, angiotensin (Table III, Group VIII) raised preexposure blood pressure but did not eliminate radiation-induced hypotension. Magnitude of this response was reduced somewhat (22 ± 5%), compared to that recorded in untreated rats (Group I = 30 ± 5%). This may have been due to the reduction in sympathetic tone in these animals because the higher blood pressure would reduce baroreceptor stimulation. Further studies are required to confirm this hypothesis of radiation-induced decrease in sympathetic tone. This effect must be relatively brief because cardiovascular function recovered within 2 to 3 min postexposure.

The highly significant correlation between mean arterial pressure and change in the log of epinephrine is consistent with the hypothesis that radiation led to a selective increase in adrenal output. A generalized increase in sympathetic activity should have also led to a parallel increase in plasma norepinephrine; however, this was not observed until 10 min postexposure (Table II). If this were a reflex-related increase in epinephrine, then levels should have fallen by 10 min postexposure by which time mean arterial pressure had recovered; however, this did not occur, and plasma epinephrine remained elevated for at least 30 min (Table II). This relationship between blood pressure and plasma epinephrine is recorded in rats after hemorrhage (*35*). Plasma epinephrine fell as blood pressure recovered. Results of the present study are more consistent with the hypothesis that radiation initiated a mechanism(s) that directly or indirectly increased the adrenal output of epinephrine. An alternative explanation for the increase in plasma epinephrine might be that there is an impairment in the enzymatic pathway responsible for catecholamine breakdown. Although the present study does not directly address this possibility, the fivefold increase in epinephrine during the first minute after the onset of R strongly suggests that this response is due to an increased release, because this sample is drawn from the arterial side and most of this blood has not yet passed though the liver (the primary site of breakdown for circulating catecholamines).

Should the cause for the cardiovascular responses to radiation involve changes in autonomic neural activity, the question remains concerning how exposure initiates

these events. Since exposure of either the head or abdominal regions results in cardiovascular responses comparable to those recorded after whole-body exposure (Table I), it is apparent that these responses are initiated by the release of factors from the irradiated tissues and that sufficient amounts are released even with partial-body radiation to cause a maximal change in the involved pathways. Stores of the factor(s) appear to be completely depleted by one exposure, and based on results obtained from repeated whole-body exposures, these stores are not reconstituted for at least 30 min. Once again, it is doubtful that mast cell stores of histamine are involved, because 48/80 can still initiate both hypotension and an increase in plasma histamine at a time when radiation fails to cause cardiovascular dysfunction.

With regard to the changes in respiratory function of irradiated rats, it is readily apparent that radiation causes a consistent decrease in rate for up to 10 min postexposure. Episodes of dyspnea and apnea during radiation-induced hypotension were recorded on several occasions and were most consistent at the highest doses (40,000 rad) and dose rates (1200 rad/sec). Despite the significant decrease in rate, hypoxia and acidosis did not develop after rats received 10,000 rad. Although this was not evaluated quantitatively, there did appear to be an increase in the depth of respiration (Fig. 3B). The later change was sufficient to maintain adequate gas exchange. Pretreatment with diphenhydramine abolished the decrease in respiratory rate, and these animals did not experience any dyspneic or apneic episodes; however, these experiments were not conducted under conditions where maximal respiratory dysfunction might have been expected (dose rate for these experiments was 180 rad/sec). Therefore, further studies are needed to clarify the protective effect of these agents for maintenance of postexposure respiratory rate.

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